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Ribosome in growth, differentiation and dedifferentiation

Jayasree Roy CHOWDHURY

Department of Tumour Biology, Chittaranjan National Cancer Research Centre, Calcutta-26

There are several evidences which have provided information pertaining to the occurrences and distributions of ribosomes. These informations reveal that ribosomes exist in two topographic situations either bound to the membranes of endoplasmic reticulum or scattered fielding the cytoplasmic matrix[1~5]. In recent years it has been shown that the functional significance of ribosomes is directly associated with the synthesis of various types of protein molecules required for the varied functions of different cell types[6~12]. It has been noted that in most of the differentiated tissues, the ribosomes are in the bound form[13~14]. It has been implicated that ribosomes in such bound form participate in the synthesis of proteins required for varied cell functions of adult tissue. Furthermore ribosomes have been shown to have profound specificity with respect to various *m*-RNA molecules in relation to protein synthesis[15~18]. Such observations provide an insight pertaining to the exploration of occurrences and distributions of ribosomes in embryonic, neoplastic and adult differentiated tissues. In my previous publications biochemical observations have provided significant evidences regarding ribosomal patterns and the effect of ions on ribosomes in the above tissues[19~21]. The present communication adds further evidences with respect to ultrastructural study regarding the topographic situations of ribosomes of normal and cancer cervix in line with my previous biochemical reports.

MATERIALS AND METHOD

Specimens of carcinoma of the cervix uteri were collected from Chittaranjan Cancer Hospital with biopsy report of epidermoid carcinoma-grade III. Normal tissues of uterine cervix were obtained from patients undergoing hysterectomy operation at Chittaranjan Seva Sadan. Histological studies of the normal cervical specimens were done to eliminate any pathological lesions. Human embryos of ten to twelve weeks gestation were collected from labour room of Chittaranjan Seva Sadan. Spontaneous abortion cases for cause apparently not in foetus were selected. Any case with association of sepsis was eliminated from the series.

ISOLATION AND SEPARATION OF BOUND AND FREE RIBOSOMES

The isolation of microsome and further fractionation into bound and free ribosomes were done as described in details previously[19]. The homogenate was prepared in Hoagland's Medium A[22] and differential centrifugation technique described by Henshaw *et al.*[13] was adopted with minor modifications. After centrifuging down the nucleus and mitochondria, the supernatant was spun at 145,000 g in No. 40 rotor of Spinco Model L Ultracentrifuge for three hours. The microsomal fraction thus obtained was layered over linear gradient sucrose solution and centrifuged in SW39 head with swinging head bucket at 40,000 g for $2\frac{1}{2}$ hours. At the end of the period of density gradient centrifugation, six drop fractions were collected from pin hole made at the bottom of the tube and the different fractions analysed at 260 mμ extinction coefficient of RNA and at 280 mμ for that of protein in Hilgers' UVISPECK Spectrophotometer.

ELECTRON MICROSCOPIC STUDIES

For the ultrastructural studies of normal and cancer cervix uteri, the specimens were fixed primarily in 6.5% glutaraldehyde and subsequently in osmium tetroxide. Further processing was done according to Wohlfahrt-Bottermann[23]. After washing in Palades' isotonic solution the tissues were dehydrated by passing them through graded alcohol and finally embedded in Epon as described by Hayward[24].

Sections were cut with the help of glass knife mounted on L.K.B. ultratome and with the aid of a dissecting microscope, were mounted in grids filmed with 1% collodion in amyl acetate. The sections which were originally stained with uranyl acetate during dehydration of tissue bits were double stained subsequently with lead citrate solution of Reynolds[25]. The sections were observed under the Siemens Elemiscope I at 8,000; 10,000 and 20,000 resolutions.

RESULTS

The biochemical evidences of the proportions of bound and free ribosomes obtained from normal uterine cervix by density gradient centrifugation and the influence of Mg^{++} and K^{+} in such isolation process have been shown in Figures 1–3[19]. The proportion of bound ribosome as shown in the third tube or the interphase of 50% and 20% sucrose, appears to be much greater than that of free ribosome seen around thirteenth tube (Fig. 1). The critical concentration of Mg^{++} for effective separation of

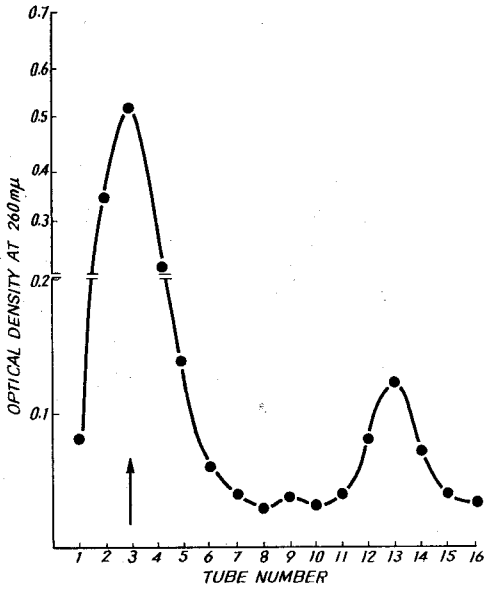

Fig. 1. Sucrose density gradient centrifugation analysis of the microsomal fraction from human normal cervix uteri. Tube number 1 is from the bottom of the gradient and the interphase of 50% and 20% is located at about tube 3 (arrow) where the bound ribosome peak is obtained. The peak at 13th tube shows the free ribosome.

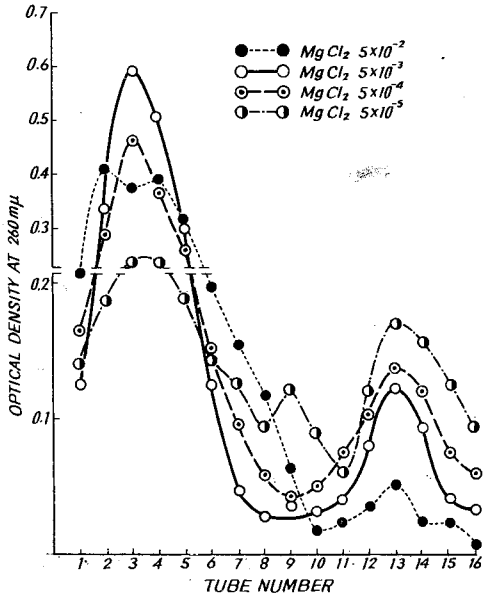

Fig. 2. Influence of different concentration Mg^{++} on isolation of bound and free ribosomes of human normal cervix uteri.

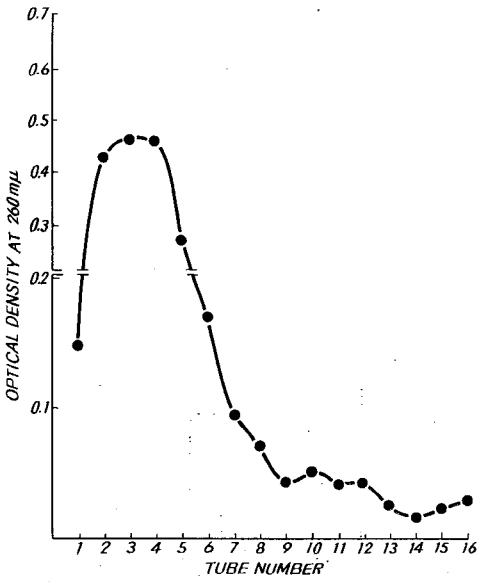

Fig. 3. Effect of omission of K^+ on isolation of bound and free ribosomes of human normal cervix uteri.

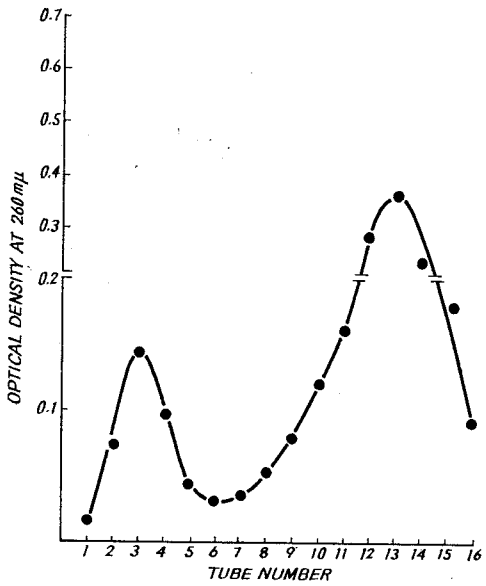

Fig. 4. Sucrose density gradient centrifugation analysis of the microsomal fraction of human cancer cervix uteri.

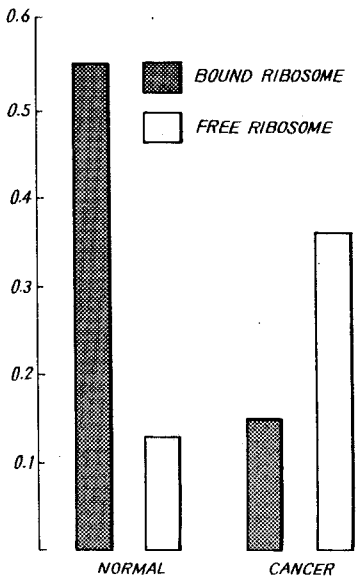

Fig. 5. Relative occurrences of bound and free ribosomes in human normal and cancer cervix uteri.

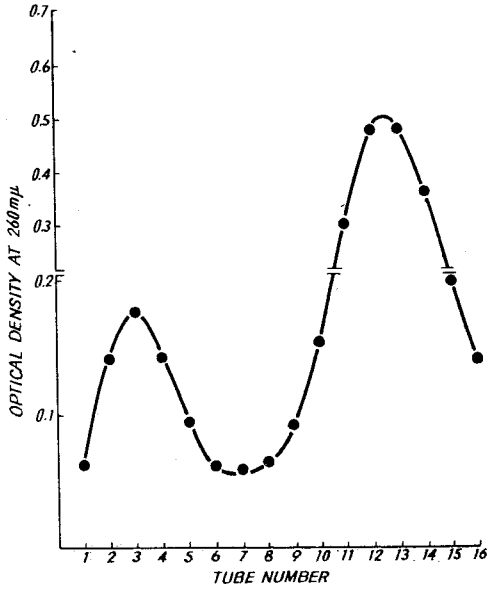

Fig. 6. Sucrose density gradient centrifugation analysis of the microsomal fraction of human embryo.

ribosomes was seen to be 5×10^{-3} M for this tissue (Fig. 2). While increase of this concentration caused a tendency for aggregation, however lowering of concentration was followed by decrease of the proportion of bound ribosome and increase of free ribosome. Figure 3 depicts the effect of omission of K^+ from the isolating media. A reverse influence was seen with respect to K^+ where omission of this ion produced aggregation of ribosomes similar to that produced by high concentration of Mg^{++}.

The subfractionation of microsome from carcinoma cervix resulted an interesting picture of bound and unbound ribosomes[20]. The ribosomes were mainly in free form compared to very low concentration of bound ribosome (Fig. 4). The relative proportion of bound and free ribosomes in malignant cervix were reverse of what was found for normal cervix (Fig. 5.) The embryonic tissue exhibited ribosomes to be mainly in free form (Fig. 6) similar to that seen for malignant condition.

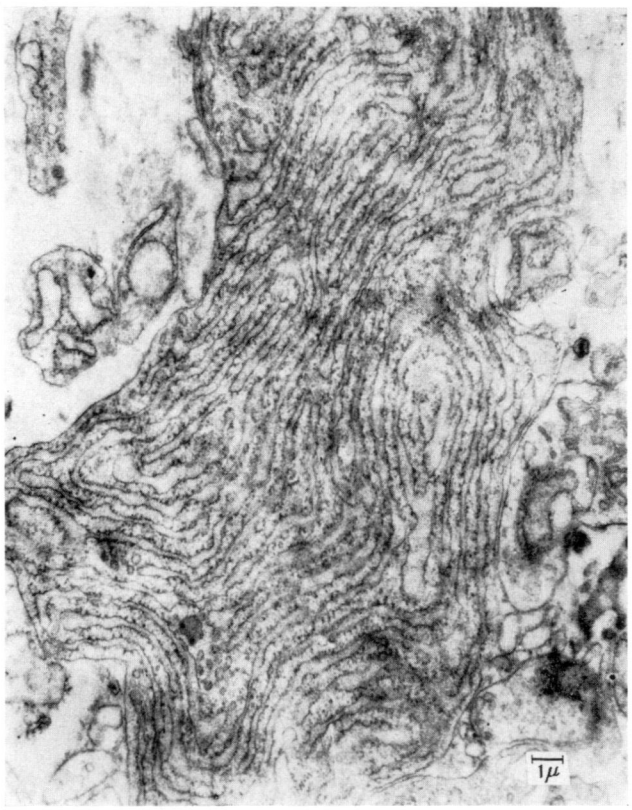

Fig. 7. Electron micrograph of human normal epithelial cell of cervix uteri. Densely packed lamellar type of ergastoplasm is seen with ribosome in the bound state. Magnification ×700,000.

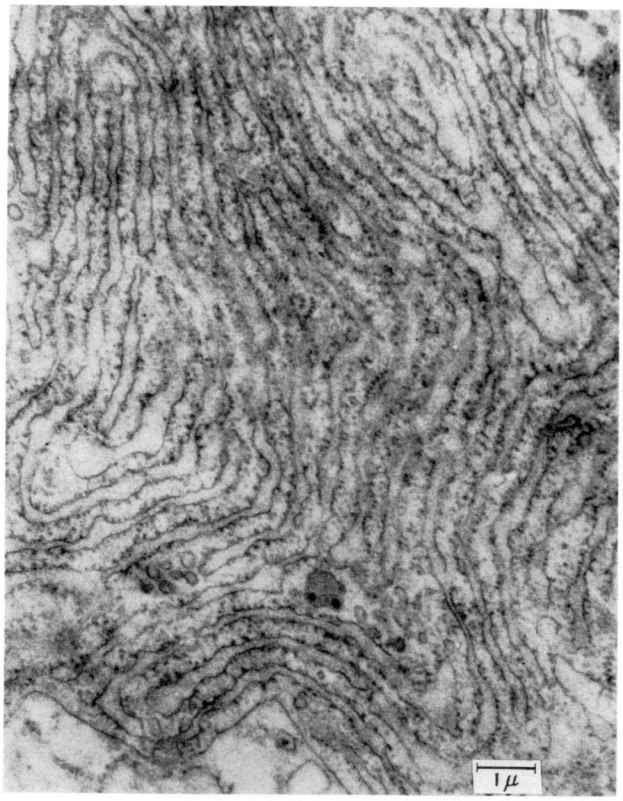

Fig. 8. Higher magnification of Figure 7 showing clearly the architecture of ergastoplasm. Magnification 140,000×.

The cytoplasmic structure when visualized under the electron microscope exhibited well developed membraneous system in normal cervical epithelium. The membranes of ergastoplasm of precornified and cornified cells show lamellar type of arrangement which are densely packed and running parallel to each other with formation of reticulosis (Fig. 7 and 8). The ribosomes are mainly in the bound form being attached to the outer border of the membraneous tubules. In basal and parabasal type of cells, the ergastoplasm is similarly arranged except the membranes seem to be less packed. The ribosomes are still in bound form with some amount of free ones lying in the cytoplasmic matrix.

The attachment of ribosomes to the mmbranes is fairly uniform maintaining approximately constant distance among them. The bound ribosomes appear to be in groups of a few, clustered together as against the free ribosomes which appear to be discrete.

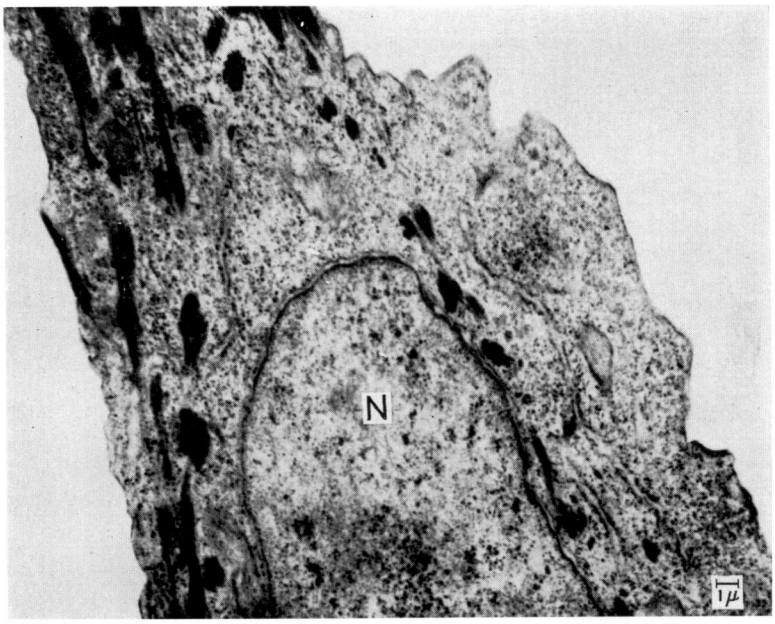

Fig. 9~11. Electron micrograph of cancer cell of human carcinoma cervix uteri. The entire cytoplasmic matrix is seen to be studded with free ribosomes with poorly developed membraneous system. Fig. 9 & 10 Magnification 56,000×. Fig. 11 & 12 Magnification 40,000×. N=nucleus.

In case of malignant cells of cervix, most of the ribosomes occur in free state and almost filling up the cytoplasmic matrix (Fig. 9–12). The membranes of the endoplasmic reticulum were rather meagre and poorly developed. These membraneous tubules are dilated irregularly the diameter of the tubules varying from 903 Å to 2520 Å as against the range of 20 Å to 1,620 Å in normal cells. The thickness of the membranes in malignant cells varied from 40 Å to 300 Å while the variation range of such in normal cell is 40 Å to 100 Å. This disorganization of the ergastoplasmic architecture appears to be typical of malignant cells of cervix. The uniformity of space between the attached ribosomes is lost. The free ribosomes of malignant cells which are studded in the cytoplasmic matrix seem to exist in aggregates. Various shapes and sizes of aggregates comprising of various numbers of free ribosomes appear to form incomplete or complete polyribosomes. However there is not much difference in the size of ribosomal particles in normal and cancer cells—the mean value being 202 Å in normal cell and 212 Å in cancer cell.

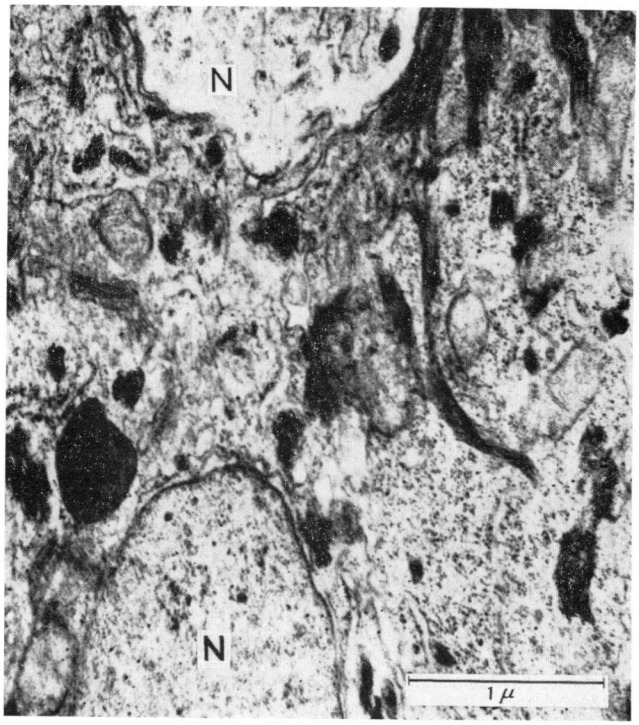

Fig. 10.

DISCUSSION

The results of the present investigation distinctly reveal that in adult differentiated tissue of cervix, the endoplasmic reticulum is well developed and the ribosomal aggregates mostly occurring in the bound form. Contrary to this, the ribosomal particles are studded in free form in the cytoplasmic matrix of both embryonic and malignant tissues. The structural integrity of the membraneous system in such tissues is not well distinguished and seems to be smooth. The ionic stress in the loosening or unfolding of ribosomes from the membranes has been noted in the differentiated tissue. In this connection it may be pointed out that the importance of ionic environment in maintaining the structural stability of ribosomes has been reported by many workers[5,26~27]. There are also evidences that the absence of Mg^{++} helps in the loosening of compact form of ribosomes and their subunits and the quarternary structure of the ribosomes is affected by the ratio of univalent to bivalent cations[27~28]. If ionic balance particularly Mg^{++} maintains the integrity of ribosomes along with the membranes in the differentiated tissues, the existence of

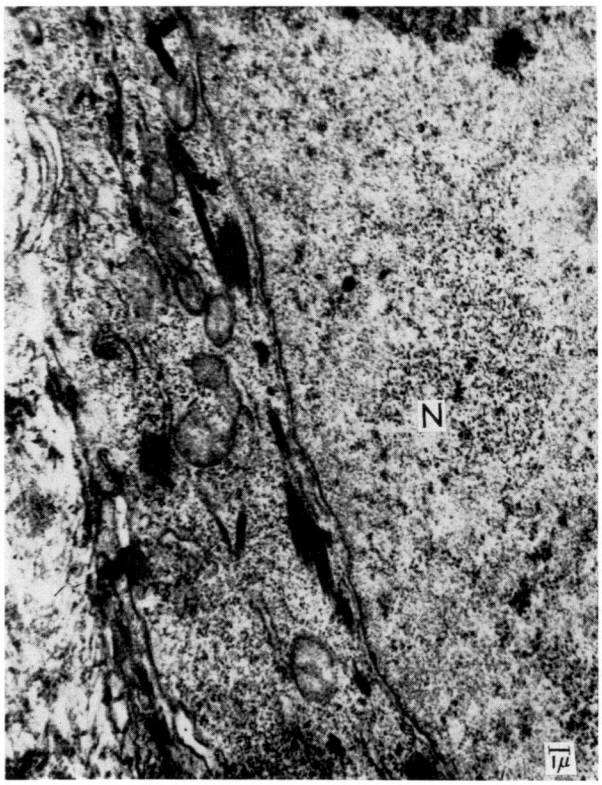

Fig. 11.

most of the ribosomes mostly in the free form in the tumour tissue assume significance regarding the critical concentration of such ionic balance in the loss of organizing ability of ribosomes along the membranes.

The central role of polyribosome in protein synthesis has been well established. There are evidences that ribosomes have selective recognition for different types of messenger RNA. Such recognition of the specificity of ribosomes in binding and translating particular RNA species has been implicated in different cell functions[18]. However in the present result, a distinct parallelism has been noted with respect to the ribosomal patterns between embryonic and malignant tissue contrary to that in normal adult cervical tissue. In this connection, it may be pointed out that a class of ribonucleoprotein particles has been identified which appear to be bound to membranes and filaments of cells. Such RNP particles have been suggested to be linked up with the process of keratinizaion of epithelial tissues[11] or collagen synthesis[10]. The arrangement of most of the ribosomes along the endoplasmic reticulum of cervical epithelium is a step by

Fig. 12. Electron micrograph of cancer cell of human carcinoma cervix uteri showing dilated and disorganised pattern of ergastoplasm. Magnification 40,000×. N=nucleus.

step acquisition as a result of differentiation concomitant with the cessation of proliferative potentialities. Very recently it has been reported that malignant cells show two types of neoantigens—one is the characteristics of malignant tissues, and the other very specific for embryonic tissues[29]. The similarities of the ribosomal patterns between embryonic and malignant tissues have been noted in the present result. However the lack of such intrinsic differences in the distribution of ribosomal particles does not decidedly signify equality or identity of embryonic and malignant tissue. Differentiation is not a state that comes out suddenly and it has evolved from the embryonic tissues through multistage processes either by acquisition or by alteration of the protein forming system of the cell. Considering all the above evidences along with present result, selective recognition of specific genetic messages by ribosomes varies with normal and abnormal cell functions. Since proteins are the end products of the genome and different protein molecules are associated with different cell functions and because of ribosomal specificity, the finding of discrepancies of ribosomal

patterns is of considerable interest in the taxonomy and evolution of organism and may have great importance in the mechanism of cell differentiation.

SUMMARY

The topographic distribution of ribosomes was investigated by electron microscopy as well as by biochemical parameters in normal adult cervical tissue, carcinoma cervix uteri and human embryo. The interaction of the ribosomes and their subparticles with endoplasmic reticulum has been studied under various ionic conditions. Specific ionic balance, particularly Mg^{++} is essential for the integrity of ribosomes along the endoplasmic reticulum.

Ultrastructural and biochemical studies show a clear cut difference pertaining to the occurrences and distributions of ribosomes. All differentiated epithelial tissues investigated in the present system exhibit that most of the ribosomes are present in bound form. Contrary to this, ribosomal particles in the malignant tissues as well as in embryonic tissues exist mostly in free forms in the cytoplasmic matrix. Such specific organization of ribosomal particles along the well developed endoplasmic reticulum of adult differentiated tissue assume significance regarding the development of protein synthesizing system in line with the differentiation phenomenon. Such intricate development of protein synthesizing system further suggest correct translation of genetic messages by selective recognition by ribosomal particles.

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REFERENCES

1. PALADE, G. E. and SIEKEWITZ, P.: *J. Biophys. Biochem. Cytol.* **2** (1956), 171.
2. HANDLER, R. W.: Protein Biosynthesis and Mebrane Biochemistry, John Wiley and Sons, New York, 1968, p. 300.
3. LOEB, J. N., HOWELL, R. R. and TOMKINS, G. M.: *J. Biol. Chem.* **242** (1967), 2069.
4. BLOBEL, G. and POTTER, V. R.: *J. Mol. Biol.* **26** (1967), 279.
5. KHAWAJA, J. A.: *Biochem. et Biophys. Acta*, **254** (1971), 117.
6. WARNER, J. R., REICH, A. and HALL, C. E.: *Science*, **138** (1962), 1977.
7. SPYRIDES, G. P. and LIPMANN, F.: *Proc. Natl. Acad. Sci. U.S.* **48** (1962), 1977.
8. GILBERT, W.: *J. Mol. Biol.* **6** (1963), 374.
9. WATSON, J. D.: *Science*, **140** (1963), 17.
10. KRETSINGER, R. H., MANNER, G., GOULD, B. S. and RICH, A.: *Nature* **202** (1967), 438.

11. FRIEDBERG, I. M.: *Biochim et Biophys. Acta* **224** (1970), 219.
12. BUTLER, J. A. V., GODSON, G. N. and HUNTER, G. D.: Cited in Protein Biosynthesis (ed. R. J. C. Harris), 1961, p. 349.
13. HENSHAW, E. C., BOJERSKI, T. B. and HIATT, H. H.: *J. Mol. Biol.* **7** (1963), 122.
14. CHAUVEAU, J., MONLÈN, Y., ROUILLER, C. and SCHNEEBEL, J.: *J. Cell. Biol.* **12** (1962), 17.
15. LODISH, H.: *Nature* **224** (1969), 867.
16. HSU, W. T. and WEISS, S. B.: *Proc. Natl. Acad. Sci. (U.S.)*, **64** (1969), 345.
17. SCHWEIGER, M., HERRLICH, P. and ZILLIG, W.: *Z. Physiol. Chem.* **350** (1969), 775.
18. NAORA, H. and KODAIRA, K.: *Biochem. et Biophys. Acta.* **224** (1970), 498.
19. ROY CHOWDHURY, J.: *Ind. Jour. Exptl. Biol.* **7** (1969), 86.
20. ROY CHOWDHURY, J.: *Science and Culture*, **34** (1968 a), 99.
21. ROY CHOWDHURY, J.: *Bull.* Chittaranjan Cancer Hosp. and Chittaranjan National Cancer Res. Centre, **6** (1968 b), 24.
22. HOAGLAND, M. B., STEPHENSON, M. L., SCOTT, J. F., HECHT, L. I. and ZAMECNIK, P. C.: *J. Biol. Chem.* **231** (1958), 241.
23. WOHLFART-BOTTERMANN, K. E.: Naturwissenschaften, **45** (1957), 140.
24. HAYWARD, A. S.: Comptes Rendus des Travaux du Laboratoire Carlxberg **33** (1963), 535.
25. REYNOLDS, E. S.: *J. Cell. Biol.* **17** (1963), 208.
26. PETERMANN, M. L.: The Physical and Chemical Properties of Ribosomes, Elsevier Publishing Company, Amsterdam, 1964, p. 134.
27. SPIRIN, A. S.: The ribosome, Springer-Verlag, Berlin-Heidelberg, 1969, p. 69.
28. SCHLESSINGER, D., MARCHESI, V. T. and KWAN, B. C. K.: *J. Bacteriol.* **90** (1965), 456.
29. BALWIN, R. W., GLAVES, D. and VOSE, B. M.: International Journal of Cancer **10** (1972), 233.